

04. April 2008

HOFFMANN · EITLE • Postfach 81 04 20 • D-81904 München

European Patent Office  
80298 Munich

Munich, April 4, 2008

**Our Ref.: 127 486 n1/n12  
3<sup>rd</sup> Party Observations J. EP 04 803 223.9**

Regarding the examination procedure of the European patent application No. 04803223.9 with the publication number EP 1689875 A1 (WO 2005/054489 A1),

**MANUFACTURE OF AMIDES**  
**(Ciba Specialty Chemicals Water Treatments Limited)**

hereafter referred to as "the present application", we herewith submit third party observations under Article 115 EPC.

We observe that the claimed subject matter of present application is not patentable under the provisions of Article 54(2) or Article 56 of the European Patent Convention because of the disclosure of the following Documents D3 and D4 and the common technical knowledge at the priority date of the present application.

**D3: EP Patent Publication No. 0243966 A2**

**D4: JP Patent Publication (Kokai) No. 2003-144144 A**

[1] nicht Patentanwalt/not German Patent Attorney  
[2] nicht/not European Patent Attorney  
[3] nicht/not European Trademark Attorney  
[4] Dutch Patent Attorney  
[5] Patentanwalt/German Patent Attorney  
[6] nicht/not British Patent Attorney

**PATENTANWÄLTE · MÜNCHEN**  
EUROPEAN PATENT ATTORNEYS  
EUROPEAN TRADEMARK ATTORNEYS  
Klaus Föhrle, Dipl.-Ing.  
Bernd Hansen, Dr., Dipl.-Chem.  
Klaus Görg, Dipl.-Ing.  
Rainer Zangs, Dipl.-Ing.  
Matthias Kindler, Dr., Dipl.-Chem.  
Roy D. Marsh, M.A. (Oxon) C.P.A. [1]  
Christiane Stein-Dräger, Dr., Dipl.-Chem.  
Detlev A. Pust, Dr., Dipl.-Phys.  
Veit Frank, Dipl.-Ing., M.Sc. (London)  
Leo Pöhl, Dr., Dipl.-Chem.  
Christopher Furlong, B.E. (U.C.D.) C.P.A. [1,3]  
Thorsten Bausch, Dr., Dipl.-Chem.  
Thomas Koch, Ph.D. (London), Dipl.-Ing.  
Friedrich Hieber, Dipl.-Phys. [1,3]  
Joseph Taormino, Dr. (I.H.U.) [1,3]  
Peter Klusmann, Dr., Dipl.-Chem.  
Georg Siegert, Dr., Dipl.-Phys.  
Peter Schweighart, Dr., Dipl.-Ing.  
Stefan Mayrhofer, Dr., Dipl.-Ing.  
Matthias Wolf, Dr., Dipl.-Chem.  
Klemens Stratmann, Dr., Dipl.-Chem.  
Peter Wiedemann, Dipl.-Ing.  
Lorenz von Kurowski, Dr., Dipl.-Ing.  
Joachim Renken, Dr., Dipl.-Chem.  
Jan-Hendrik Spilges, Dr., Dipl.-Chem.  
C. Thomas Becher, Dipl.-Ing.  
Elisabeth Engelhard, Dr., Dipl.-Biol.  
Martin Bachefin, Dr., Dipl.-Chem.  
Georg M. Kreuz, Dipl.-Ing. [1,3]  
Stephan Däser, Dr., Dipl.-Chem.  
Ulrike Ciesla, Dr., Dipl.-Chem.  
Oliver Dannenberger, Dr., Dipl.-Chem.  
Kathrin Föhrle, Dr., Dipl.-Chem.  
Anne K. Schön, Dipl.-Ing.  
Ulrich Kross, Dipl.-Ing. [2]  
Henrik Vocke, Dr., Dipl.-Ing.  
Andreas Görg, Dipl.-Ing., DIP (FPLC)  
Philipp Nordmeyer, Dipl.-Phys.  
Frank van Bouwelen, Ph.D. (Cantab), M.Sc. [1,4]  
Lüder Behrens, Dr., Dipl.-Biol. [2]  
Morten Garberg, M. Chem. (Oxon) C.P.A. [1,3]  
Klaus Breitenstein, Dr., Dipl.-Chem. [2]  
Markus C. Müller, Dr., Dipl.-Phys., M.Sc. [2]  
Karen Thirwell, B.Sc. (Bristol) C.P.A. [1,3]  
Bianca-Luca Vos, Dr., Dipl.-Chem.  
Andreas Steffert, Dr., Mag.rer.nat. (Biol.)  
Christine Streege, Dr., Dipl.-Ing. [1,3]  
Nicolas Douxchamps, M.Sc. [1,3]  
Greg Sach, B.Sc. (U.C.L.) [1,3]  
Steffen Thomas, Dr., Dipl.-Chem. [2]

**RECHTSANWÄLTE · MÜNCHEN**  
ATTORNEYS AT LAW  
EUROPEAN TRADEMARK ATTORNEYS  
Güntram Rahn, Dr. iur.  
Wendy v.d. Osten-Sacken, LLM.  
Holger Strämann  
Anja Petersen-Padberg, Dr. iur.  
Alexander González, Dr. iur.  
Christian Holger Folz, Dr. iur.  
Dirk Schössler-Langehelme, Dr. iur.  
Ortrun Günzel  
Marc Demauer, Dr. iur., LLM.  
Angela Wenninger  
Clemens Tobias Steins, Dr. iur., LLM.  
Isabell Kuschel, LLM.  
Oliver Sude

**BRITISH PATENT ATTORNEYS · LONDON**  
EUROPEAN PATENT ATTORNEYS  
EUROPEAN TRADEMARK ATTORNEYS  
Stephen J. Avery, M.A. (Oxon)  
David J. Lethem, M.A. (Cantab) [3]  
Alan Mitchell, M.A. (Oxon) [3]  
Susan M. Dupuy, M.Sc. (London)  
Alistair Russell, M.Eng. (Cantab) [3]  
David Sproston, B.Sc. (London)  
John F. Hardwick, B.Sc. (Hons)  
Sabine Böhm, Ph.D. (London), B.Sc. [5,6]

**CONSULTANTS · MÜNCHEN**  
Karl Kohlmann, Dipl.-Ing.

**MÜNCHEN**  
Arabellastrasse 4  
D-81925 München  
Fax +49 (0)89-918356  
Telefon +49 (0)89-92409-0  
nm@hoffmann-eitle.com

**LONDON**  
Sardinia House, Sardinia Street  
52 Lincoln's Inn Fields  
London WC2A 3LZ  
Phone London +44 (0)20 7404 0116  
www.hoffmann-eitle.com

In addition, this subject matter is also not patentable under Article 54(2) because of the disclosure of D1 and D2, as pointed out in the examination report dated September 20, 2006. The disclaimer, which was introduced by the applicant in his submission of January 18, 2007 in order to provide a distinction over D1 and D2, is unclear and not admissible. Moreover, this disclaimer does not even render the claimed subject matter novel over the comparatives examples of D1.

### 1. Subject Matter of Claim 1 of the present Application

The subject matter of claim 1 of the present application as filed, displayed in terms of a feature analysis, relates to:

- (A): *A method of producing an amide from the corresponding nitrile comprising the following steps,*
- (B): *i) providing a microorganism capable of producing a nitrile hydratase biocatalyst,*
- (C): *ii) culturing the microorganism in a growth medium,*
- (D): *iii) storing the microorganism,*
- (E): *iv) contacting the nitrile with the microorganism in an aqueous medium and thereby converting the nitrile to the amide,*
- (F): *wherein the microorganism is stored as none actively growing free cells in a storage medium that comprises water*

In his submission of January 18, 2007, the applicant has introduced the following disclaimer:

- (G): *and with the proviso that the storage medium is neither*
  - (a) a neutral or weakly basic aqueous solution of inorganic salts, having a molarity ranging from 100mM to the saturation concentration of the inorganic salts,*
  - (b) nor an aqueous solution of at least one compound selected from the group consisting of inorganic salt(s) and inorganic bicarbonate salt(s)*  
*wherein the total concentration of the inorganic salt(s) ranging from about 100mM to the saturation concentration of the inorganic salt(s).*

According to this submission, this disclaimer (which is not supported by the application as filed) is intended to provide distinction over the documents D1 and D2. These documents,

which belong to the same technical field as the present application, constitute prior art under Article 54(2) EPC.

## 2. Non-admissibility of the Disclaimer

In his response, the applicant states that this disclaimer restores novelty over an accidental anticipation and therefore should be allowable. In order to support this statement, the applicant merely argues that D1 and D2 do not suggest a surprising increase of enzymatic activity during storage, which is, allegedly, characteristic for the invention of the present application. Hence, these documents would not be relevant for inventive step of the claimed subject matter, and thus constitute an accidental anticipation.

This argument, however, does not hold, as follows. The necessary criterion for accidental anticipation is that *the disclosure in question is so unrelated and remote to the claimed invention that the person skilled in the art would never have taken it into consideration when making the invention* (Part C, chapter VI, section 5.3.11, see also T 14/01 and T1146/01). This criterion is not met in case of D1 and D2, as these documents are concerned with essentially the same subject matter as the present application. These documents also relate to microorganisms such as *Rhodococcus*, which are capable of producing nitrile hydratase, and to processes for producing amides such as acrylamide out of the respective nitriles. In particular, as described on page 3, line 26 to page 4, line 3 of the present application, these documents are concerned with the storage of the microbial cells while preserving their enzymatic activity, just like the present application.

Therefore, these documents relate to the same problem as the present application and propose a solution that falls under claim 1 of the present application. Thus, the anticipation is not accidental, and the disclaimer is not allowable.

In addition, the disclaimer is unclear. In particular, the term "weakly basic" has no well-defined meaning in terms of a specific pH range. In addition, it is not clear whether the molarity of 100mM relates to the concentration of salts in total, whether at least one single species of salt is to be present in 100mM or more, or whether all salts present in the solution should be present in 100mM. Finally, dependent claim 3 covers the physiological saline to be used as storage medium, which has "neutral" pH and comprises 0.9 % w/v NaCl (corresponding to concentration of about 155mM NaCl). In case of the physiological

buffer solutions of claim 3, the situation is similar (e.g., 1x PBS, 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH of 7.4). Thus, the media of claim 3 fall under the disclaimer, rendering the scope of protection unclear.

In summary, the undisclosed disclaimer (G) is not allowable under the EPO examination guidelines. Moreover, taking the comparative examples of D1 into account, the disclaimer does not even render the claimed subject matter new (see, e.g. page 8, line 35-40 of D1). Therefore, we believe that the disclaimer may be safely ignored when assessing the novelty of the claimed subject matter in view of D3.

### **3. Lack of Novelty of the Subject Matter of Claim 1 in view of D3**

D3 discloses the combination of features (A)-(F) of claim 1 of the present application and thus is prejudicial for the novelty of the subject matter of this claim, as shown in the following.

In particular, page 2, lines 19-23, of D3 reads:

*The microorganisms used in the present invention are those of the genus *Pseudomonas* capable of producing nitrile hydratase and hydrating nitriles, especially acrylonitrile, to produce the corresponding amides, especially acrylamide.*

According to this passage, D3 is concerned the use of microorganisms having ability to produce nitrile hydratase, in order to produce the corresponding amides from nitriles. This corresponds to the disclosure of features (A) and (B).

Of course, these microorganisms of D3 are cultivated in a growth medium. In this respect, lines 1-10 of D3 state:

*Cultivation of these microorganisms is ordinarily carried out under aerobic conditions by inoculating strains of the respective microorganisms into culture media containing: carbon sources such as glucose, ...”*

Therefore, D3 discloses feature (C) in combination with (A) and (B) as well.

D3 also describes the storage of these microorganisms. In particular, page 5 of D3 (lines 15-20) states that the microorganisms may be stored in the suspended state in various buffers or in a medium such as a physiological saline, while the nitrile hydration activity could be preserved during storage by adding certain stabilizers:

*The preservation of nitrile hydration activity can be attained by adding any of the above enumerated compounds to a suspension or solution of the previously mentioned microorganism cells ... dispersed or dissolved in various buffers or physiological saline.*

According to page 4 of D3, lines 17-20, these stabilizers are organic substances:

*The stabilizers used in the present invention are compounds selected from nitriles, amides, and organic acids and salts thereof, ...”*

Hence, D3 discloses feature (C) as well. In addition, when these microorganisms are stored in such media in the suspended state, they are present in the form of free cells, which, in the absence of nutrients, do not actively grow. And, of course, the storage media such as buffers or physiological saline of course comprise water. Thus, Feature (F) is also met.

In addition, the passages cited above disclose the storage in physiological saline solution or buffer solutions. The examples of D3 use physiological saline. Physiological saline is also one of the acceptable media for storing the microorganisms of the present application, and is explicitly mentioned in claim 3.

Finally, D3 also discloses the use of the cell suspension prepared and stored as described above for the preparation of an amide from the nitrile (lines 19-26, page 6 of the specification):

*In each of the following experimental examples, 0.1 ml of an enzyme solution or cell suspension was added to 9.9 ml of M/20 phosphate buffer (pH 7.7) containing 2.5% by weight of acrylonitrile, and the resulting solution was caused to react at 10°C for 10 minutes. ... acrylamide produced ...*

Clearly, this description states that the nitrile is contacted with the microorganisms in aqueous suspension, thereby converting the nitrile to the amide. Thus, Feature (E) is disclosed as well.

In summary, D3 discloses features (A) to (F) of claim 1 of the present application in combination. Therefore, the subject matter of claim 1 of the present application is not new.

#### 4. Lack of Novelty of the Subject Matter of Claims 2, 3, 5, 7 and 8 in view of D3

The subject matter of the dependent claims is not new in view of D3 as well.

##### 4.1 *Claim 2*

Claim 2 of the present application states that the microorganism should be recovered from the growth medium in the form of an aqueous paste comprising whole microbial cells. As described in the examples of D3 (see, e.g., page 7, lines 6-12), the cells are recovered from the growth medium by means of centrifugation, just like in the present application (see, e.g., examples of the present application). The product, which is directly obtained by such a procedure, will be in the form of an aqueous paste.

Therefore, the subject matter of claim 2 is not new in view of D3.

##### 4.2 *Claim 3*

Claim 3 of the present application concerns the method of claim 1, further including features (H) and (I):

(H): *the microorganism is recovered from the growth medium*

(I): *and is stored as an aqueous suspension of microbial cells in a suspending medium selected from the group consisting of water, physiological saline solution, a physiological buffer solution and an aqueous liquid containing at least one component of the growth medium.*

According to D3, the cells are also separated from the growth medium and stored as a suspension in a medium such as physiological saline (see above). In particular, the examples of D3 disclose the following (lines 6-23, page 7):

*Cells were separated from the culture fluid by centrifugation (3°C, 10,000 rpm, 20 minutes) and washed with a physiological saline. The washed cells were subjected to centrifugation under the same conditions and then suspended in a physiological saline to obtain a suspension of washed cells. [...] To 2.5 ml of this cell suspension was added [...], and then cell suspension was left standing for 5 days in ice-cooled state.*

In other words, D3 describes a technique of recovering the aforementioned microorganisms from a growth medium (H), suspending them in the form of an aqueous liquid in a physiological saline, and preserving a cell suspension of the aforementioned microorganisms (I).

Thus, D3 discloses features (H) and (I) in combination with features (A) to (F). Therefore, the subject matter of claim 3 is not new as well.

#### 4.3 *Claim 5*

Claim 5 of the present application additionally requires the amide to be an ethylenically unsaturated amide such as acrylamide or methacrylamide. This subject matter is disclosed in D3 in combination with features (A) to (F) as well (see passages cited above, page 2, lines 19-23 of D3). Therefore, the subject matter of claim 5 is not new as well.

#### 4.4 *Claim 7*

The method of claim 7 additionally characterizes the storage temperature of the microorganism as being above the freezing point of the storage medium. Temperatures above 0°C, in particular between 4 and 30°C, are deemed to be preferable. Storage at such temperatures is disclosed in D3 as well, e.g., on page 5, lines 31-34:

*While the suspension or solution thus obtained may be stored at room temperature as long as the storage period is short, storage at a low temperature, especially at a temperature in the vicinity of 0°C is preferred.*

We also refer to the passage page 7 of D3, lines 6-23, as cited above, disclosing storage of the “*suspension*” (i.e., a liquid) in the “*ice-cooled state*” (i.e., at about 0°C). In conclusion, D3 also destroys the novelty of the subject matter of claim 7.

#### 4.5 *Claim 8*

Claim 8 of the present application defines the storage period of at least 2 days, preferably between 3 and 28 days.

Such storage periods are also disclosed in D3. In particular, the examples of D3, disclose storage periods of 5 days (page 7, lines 13-23 as cited above), 4 days (page 10, lines 26-32) or 3 days (page 11, line 28 to page 12, line 2). Therefore, the subject matter of claim 8 is not new as well.

### 5. Lack of Inventive Step of the Subject Matter of Claims 4, 6, 9 and 10 in View of D3

#### 5.1 *Claim 4*

Claim 4 of the present application is directed to a method according to claim 1, in which the microorganism is retained in the growth medium. This means that, according to claim 3, the microorganism is not isolated and stored at all, but kept in the growth medium. In other words, there is no real difference between feature (C) and (D). As long as the microorganism grows, the step is considered as “culturing the microorganism” in the sense of feature (C), and afterwards, the step is considered as “storing the microorganism” in the sense of feature (D).

Starting from D3, the skilled person would have to omit the method for preserving the cells as taught therein, and use the aged and no longer growing cell culture as such. This technical difference, however, does not involve an inventive step, as it is obvious that the aged cell culture may also be used for producing amines out of the nitriles.

Moreover, this technical difference obviously results in a decreased activity of the enzyme, as the steps for the preservation thereof are omitted. An "invention" resulting from a foreseeable disadvantageous modification of the closest prior art does not involve an inventive step (T 119/82).

#### 5.2 *Claim 6*

The subject matter of claim 6 adds the requirement that the components of the growth medium comprised in the storage medium include urea or urea derivatives.

Culturing *Rhodococcus* cells in the presence of urea, however, is well known in the art (see, e.g., Example 6 of D1, page 9, line 33). Therefore, adding urea to the growth medium and the storage medium (which may, according to claim 4, be identical) is obvious for the skilled person starting from D3. In consequence, the subject matter of claim 6 lacks an inventive step view of D3 in combination with D1 or the skilled person's common knowledge.

#### 5.4 *Claim 9*

According to claim 9 of the present application, the microorganism characterized as to be of the *Rhodococcus* genus, preferably of the *Rhodococcus rhodochrous* species, whereas the microorganism used in D3 is of the *Pseudomonas* genus.

Use of *Rhodococcus rhodochrous* for the production of acrylamide out of the nitrile is well known in the art, however, and according to page 4, lines 12 of the present application, this species is also used commercially for that purpose. Therefore, it is obvious for the skilled person starting from D3 to employ *Rhodococcus rhodochrous*. Hence, the subject matter of claim 9 lacks an inventive step in view of D3 in combination with the skilled person's common knowledge.

In addition, the subject matter of claim 9 is also obvious from the combination of D3 and D4. Document D4 (translation thereof attached) relates to a method for preserving a liquid comprising a microbial mass to be subjected to a thermal sterilization treatment, wherein enzyme activity can be maintained after thermal sterilization. Specifically, D4 describes the

preservation of a liquid, which comprises microorganisms containing nitrile hydratase, characterized in that a specific pH range and aeration and stirring conditions are applied

[0006] of D4 discloses that these microorganisms may preferably belong to the *Rhodococcus* genus:

*Specifically, preferred examples of such microorganisms include those belonging to ... , genus Rhodococcus including Rhodococcus rhodochrous as a typical example, ...*

Therefore, use of *Rhodococcus rhodochrous* is obvious for the skilled person starting from D3 also in light of the teaching of D4.

In addition, documents D1 and D2 also mention the use of *Rhodococcus rhodochrous* (see examples 5 and 6 of D1, col. 1, l. 51-52 of D2). Therefore, the subject matter of claim 9 would lack inventive step in view of D3 in combination with D1 or D2 as well.

## **6. Double Patenting in View of EP1689861 (Claim 10)**

Further to the lack of novelty or inventive step of the subject matter of claims 1-9, we also observe a potential double patenting issue regarding claim 10.

Claim 10 requires the microorganism as a certain *Rhodococcus rhodochrous* with the deposit number NCIMB 41164, which is said to be particularly suitable and is described in co-pending UK application no. 0327907.2 (corresponding to EP1689861). The claimed subject matter of this document includes said microorganism (claim 1 of the A1 publication), the cultivation thereof in the presence of urea (claims 2-6), the storage thereof in the form of non-actively growing free cells in a storage medium comprising water (claims 25-27), and, finally, the use thereof in a method for producing nitrile (claims 8-12 and 30). Thus, the subject matter of claim 10 is already claimed in EP1689861.

## **7. Conclusion**

In view of the above, the subject matter of claim 1 of the present application does not only lack novelty in view of D1 and D2, but also in view of D3. The dependent claims do not

contain new and inventive subject matter as well. Therefore, the present application should be rejected under the provisions of Article 54(2) or 56 of European Patent Convention.

*K. Stratmann*

Dr. Klemens Stratmann  
European Patent Attorney  
Association No. 151

Encl.:

Copy of D3  
Copy of D4  
Translation of D4